Net portal and hepatic flux of nutrients in growing wethers fed high-concentrate diets with oscillating protein concentrations

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Net portal and hepatic flux of nutrients in growing wethers fed high-concentrate diets with oscillating protein concentrations[1,2]

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ABSTRACT: We hypothesized that oscillating dietary CP would improve N retention by increasing the uptake of endogenous urea N by portal drained viscera (PDV), compared with static dietary CP regimens. Chronic indwelling catheters were surgically implanted in the abdominal aorta, a mesenteric vein, a hepatic vein, and the portal vein of 18 growing Dorset × Suffolk wethers (44.6 ± 3.6 kg of BW). Wethers had ad libitum access to the following diets in a completely randomized block design: 1) Low (9.9% CP), 2) Medium (12.5% CP), or 3) Low and High (14.2% CP) diets oscillated on a 48-h interval (Osc). Dry matter intake was greater (P = 0.04) for the Osc diet (1,313 g/d) than the Low diet (987 g/d) and was intermediate for the Medium diet (1,112 g/d). Nitrogen intake was not different between the wethers fed the Osc (25.4 g/d) and Medium diets (22.2 g/d), but was lower ($P < 0.01$) in wethers fed the Low diet (16.0 g/d). Wethers fed the Osc diet (6.7 g/d) retained more ($P < 0.04$) N than did those fed the Medium diet (4.0 g/d). Hepatic arterial blood flow was not different (P = 0.81) between wethers fed the Osc (31 L/h) or Medium diet (39 L/h) but was greater (P = 0.05) in wethers fed the Low diet (66 L/h). Net release of α-amino N by the PDV did not differ (P = 0.90) between the Low (37.8 mmol/h) and Medium diets (41.5 mmol/h) or between the Osc (53.0 mmol/h) and Medium diets (P = 0.29). Net PDV release of ammonia N was less (P = 0.05) for the Low diet than for the Medium diet, and this was accompanied by a similar decrease (P = 0.04) in hepatic ammonia N uptake. Urea N concentrations tended to be (P = 0.06) less in arterial, portal, and hepatic blood in wethers fed the Low diet compared with those fed the Medium diet. Wethers fed the Osc diet tended (P = 0.06) to have a greater PDV uptake of urea N than did those fed the Medium diet, but there was no difference between the Osc and Medium diets (P = 0.72) in hepatic urea N release. Net PDV uptake of glutamine tended to be greater ($P < 0.07$) in wethers fed the Low diet (6.7 mmol/h) than those fed the Medium diet (2.7 mmol/h). These data indicate that oscillating dietary protein may improve N retention by increasing endogenous urea N uptake by the gastrointestinal tract.

Key words: metabolism, nitrogen, oscillation, wether

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INTRODUCTION

Concentrated animal feeding operations, such as beef cattle feedlots, are currently required to develop a comprehensive nutrient management plan to address the fate of N and P to comply with the revised National Pollutant Discharge Elimination System and effluent limitation guidelines and standards for concentrated animal feeding operations (68 Federal Register 7176; February 12, 2003). Therefore, it has become imperative for these operations to develop practices that will optimize the retention of nutrients by feedlot animals and minimize the excretion of these nutrients in feces and urine. An obvious management practice that will accomplish this is to adapt feeding practices to maximize nutrient retention.

Oscillating dietary protein concentrations of finishing rations on a 48-h basis can improve the N retention of finishing steers (Cole et al., 2003; Ludden et al., 2003) and sheep (Cole, 1999) relative to those consuming a similar amount of protein at a static concentration. However, observations of the effects of oscillating dietary CP in ruminants fed forage-based diets have been less consistent (Collins and Pritchard, 1992; Simpson et

[1]Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

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Table 1. Dry matter and chemical composition (DM basis) of the experimental diets[1]

Item	Low	Medium	High
Ingredient, %			
Cottonseed hulls	15.00	15.00	15.00
Ground corn	75.82	72.27	73.82
Soybean meal	1.00	5.48	7.45
Urea	—	0.10	0.50
Cane molasses (dry)	7.00	6.00	2.00
Limestone	1.00	1.05	1.20
Dicalcium phosphate	0.15	0.07	—
Vitamin A, D, and E premix[2]	0.01	0.01	0.01
Trace mineral premix[3]	0.02	0.02	0.02
Nutrient content			
DM,[4] %	88.00 ± 0.01	89.00 ± 0.01	88.20 ± 0.28
CP,[4] %	9.91 ± 0.22	12.50 ± 0.18	14.19 ± 0.35
RDP,[5] % of CP	48.64	53.05	57.18
ME,[5] Mcal/kg	2.88	2.88	2.88
NEm,[5] Mcal/kg	1.92	1.92	1.92
NEg,[5] Mcal/kg	1.28	1.28	1.28
Ca,[5] g/kg	4.86	4.88	4.89
P,[4] g/kg	2.60 ± 0.14	2.95 ± 0.05	2.80 ± 0.01

[1]Diets: Low (9.9% CP); Medium (12.5% CP); High (14.2% CP).
[2]The vitamin A, D, and E premix contained 8,800,000 IU of vitamin A; 880,000 IU of vitamin D; and 880 mg of vitamin E per kilogram.
[3]The trace mineral premix contained 15% Ca, 12% Zn, 8% Mn, 10% Fe, 0.2% I, and 0.1% Co.
[4]Measured values of weekly composites during the balance trials (n = 2) ± SD.
[5]Calculated from the NRC (1985) guidelines.

al., 2001; Ludden et al., 2002). Cole (1999) hypothesized that the improvement in N retention by oscillating dietary CP could be due to improvements in N recycling, quality of the intestinal protein supply, metabolic use of AA, or a combination of these factors. This may explain why there is a less consistent improvement in ruminants fed forage-based diets, which would provide less carbohydrate fermentation in the rumen than high-concentrate diets, and consequently less potential N returning to the rumen (Huntington, 1989). The objective of this study was to test the hypothesis that oscillating the dietary CP of finishing ruminants will improve N retention by altering the uptake of endogenous urea by portal drained viscera (**PDV**).

MATERIALS AND METHODS

Animals and Experimental Procedures

The US Meat Animal Research Center Animal Care and Use Committee approved these experimental procedures. Eighteen Dorset × Suffolk wether lambs (initial BW, 44.0 ± 3.4 kg) from the US Meat Animal Research Center flock were surgically fitted with chronic indwelling catheters in the abdominal aorta, a mesenteric vein, the portal vein, and a hepatic vein (Ferrell et al., 1991). The wethers were housed in individual 1.17 × 1.17-m pens at 20°C with a 14:10 h light:dark cycle.

Wethers were fed at 0800 and 2000 h daily to allow ad libitum access to feed. All diets were formulated to meet or exceed NRC (1985) recommendations for growing lambs (Table 1), with the exception of protein, which was adjusted among treatments including soybean meal. Wethers were adapted from a diet of alfalfa pellets to the finishing ration by combining the Medium diet (12.50% CP) with the alfalfa pellets by substituting an additional 25% of the diet per day in a 3-d "step-up" fashion. When the wethers were adapted to the finishing ration, they were assigned to the Low (9.91% CP; n = 5), Medium (12.50% CP; n = 5), or Oscillating [**Osc**; Low (9.91% CP) and High (14.19% CP) diets oscillated on a 48-h interval; n = 8] protein diets. Eight wethers were assigned to the Osc treatment to allow for equal representation across all days within the 4-d cycle during the measurement of nutrient fluxes. Although the diets differed in protein concentrations, all diets included the same ingredients, including soybean meal, to allow similar AA from all sources (albeit in differing amounts) to be presented to all wethers.

The wethers were allowed to adapt to each dietary regimen for at least 13 d, after which total collections of urine, feces, and orts were conducted for 4 d in portable crates (0.40-m wide × 1.2-m long × 1.17-m high). Collections were made during 2 sequential weeks, with 3 wethers from each treatment sampled during the first week and the remainder collected during the second week. A 4-d collection was used to encompass the entire feeding cycle for the Osc group. Feed was sampled daily (100 g) during the 4 d of sample collection and composited for nutrient analysis. Urine was collected in plastic bottles containing 100 mL of 6 *M* HCl. Orts, feces, and urine were collected daily, weighed, and an aliquot (100% of orts, 20% of feces, and 20% of urine daily output) was retained. To ensure a pH <4, urine pH was measured with pH-sensitive paper before collection of

aliquots. Aliquots were pooled within wether and kept frozen at <–17°C until analyzed.

Composited feed, ort, and fecal samples were weighed, dried in a forced-air oven (55°C), weighed again, and then ground with a Wiley mill (Arthur Thomas Co., Philadelphia, PA) fitted with a 1-mm screen. A subsample of feed, orts, and feces was dried at 70°C for determination of DM. Concentrations of N (LECO CN-2000 carbon/nitrogen analyzer, LECO Corporation, St. Joseph, MI) and P (HNO_3 digestion and subsequent color development using the Fiske chemical method; Fiske and Subbarow, 1925) were determined in feed, orts, feces, and urine for nutrient balance. Any N losses from feces attributable to drying before analysis were assumed to be negligible and similar across treatments, although this may have influenced the assumptions about overall N use by the wethers. Urinary urea N was measured (Marsh et al., 1965) using a Technicon AutoAnalyzer (Technicon AutoAnalyzer Systems, Tarrytown, NY).

After the total collection of urine and feces, the wethers were returned to their pens for at least 3 d to allow recovery from the balance trial. After the recovery period, blood was sampled during 2 separate periods. The sampling periods were at least 2 wk apart to allow recovery from blood loss. Two wethers on the Low diet became anorexic and were removed from the study before collection of blood samples during the first nutrient flux collection period, and 1 wether on the Low diet lost arterial catheter patency between the first and second periods. This loss of wethers on the Low treatment may have impaired the ability to detect differences associated with the Low treatment.

During the first sampling period, blood was collected from 4 wethers on the first day and from 3 wethers on each of the subsequent days, with at least 1 wether from each treatment sampled on a given day, with the exception of those on the Low treatment, which were sampled only during the first 3 d. During the second blood sampling period, 4 wethers were sampled on each of the first 3 d and 3 wethers were sampled on the fourth day. Sampling of the wethers was stratified to provide for equal representation of the different days within the protein oscillation cycle and representation of each day of the oscillation cycle within each of the 2 blood sampling periods (n = 4 for each day of the 4-d cycle). During blood sampling, the wethers were placed in portable crates (0.40-m wide × 1.2-m long × 1.17-m high) and allowed ad libitum access to feed and water. A primed (15 mL), continuous infusion (0.8 mL/min) of paraaminohippuric acid (3% wt/vol) through a 0.22-μm sterile filter into the mesenteric vein was begun at 0700 h. Blood was drawn (10 mL) into heparinized syringes simultaneously from the mesenteric arterial, portal venous, and hepatic venous catheters at 0800 h and then hourly for 6 h. Although the blood sampling did not encompass the entire day, it did encompass the time of day when the majority of the feed was consumed. Blood samples were immediately placed on ice.

Within 4 h, 0.666 mL of blood was mixed with 1.998 mL of water for analysis of paraaminohippuric acid (Harvey and Brothers, 1962), α-amino N (**AAN**; Palmer and Peters, 1969), urea N (Marsh et al., 1965), and ammonia N (Huntington, 1982) using a Technicon AutoAnalyzer System (Technicon AutoAnalyzer Systems), and blood flows and net nutrient fluxes were calculated (Ferrell et al., 1999). An additional sample of blood from each vessel was drawn anaerobically into a 1-mL heparinized syringe, placed on ice, and analyzed within 10 min for hemoglobin and percentage of oxygen saturation of the hemoglobin (Hemoximeter, model OSM 1, Radiometer, Copenhagen). Blood oxygen concentrations were calculated as described by Burrin et al. (1989). The blood in this 1-mL syringe was also immediately analyzed for glucose, L-lactate, glutamine, and glutamate using an immobilized enzyme system (Model 2700 YSI, Yellow Springs Instruments, Yellow Springs, OH).

Statistical Analysis

Data on nutrient intake and balance parameters were analyzed by ANOVA using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). The model for the completely randomized block design included week of collection and dietary treatment as fixed effects and lamb within treatment as a random effect. Two single-degree-of-freedom contrasts were made to evaluate the dietary treatment effects on the balance trial variables: 1) Medium vs. Osc and 2) Low vs. Medium.

Arithmetic means for metabolite concentrations in whole blood, blood flow, and net flux of metabolites across the PDV and liver were calculated for each individual day of sampling and analyzed by ANOVA using the MIXED procedure of SAS. The Osc treatment was separated into measurements made when the wethers were consuming the High diet (**OsH**) or the Low diet (**OsL**). The model for the completely randomized block design included week of collection and dietary treatment as fixed effects and animal within treatment as a random effect. Three single-degree-of-freedom contrasts were made to evaluate the dietary treatment effects: 1) OsH vs. OsL, (2) Medium vs. Osc, and (3) Low vs. Medium. A difference between dietary treatments was declared when $P \leq 0.05$, and a tendency for dietary treatments to differ was declared when $0.05 < P \leq 0.10$.

RESULTS

The diets used in this study were originally formulated to be similar in chemical composition, with the exception of protein concentration. For this reason, soybean meal was also included in the Low treatment to ensure that all diets would contain AA from similar sources, although the amounts would differ. Although subsequent analysis (Table 1) of the diets demonstrated that we were successful in altering the CP concentra-

Table 2. Nutrient intake, digestion, and retention of wethers fed a high-concentrate diet with low, medium, or oscillating (Osc) dietary protein[1]

Item	Treatment			SEM[2]	Contrast, *P*-value	
	Low	Medium	Osc		Medium vs. Osc	Low vs. medium
No. of wethers	5	5	8			
DM						
DMI, g/d	987	1,113	1,313	105	0.17	0.42
Apparent DM digestibility, %	70.0	72.0	70.9	2.43	0.73	0.57
Nitrogen						
N intake, g/d	16.0	22.2	25.4	1.89	0.22	0.04
Feces N, g/d	8.2	9.2	10.8	1.04	0.24	0.52
Urine N, g/d	4.6	9.1	7.9	0.89	0.32	0.01
Urine urea N, g/d	2.1	4.8	4.8	0.38	0.93	0.01
Apparent N digested, g/d	7.8	13.0	14.5	0.98	0.26	0.01
N retained, g/d	3.2	4.0	6.7	0.90	0.04	0.56
Phosphorus						
P intake, g/d	2.6	3.4	3.6	0.29	0.52	0.09
Feces P, g/d	2.1	2.8	2.7	0.27	0.84	0.09
Urine P, g/d	0.18	0.25	0.20	0.14	0.80	0.75
Apparent P digested, g/d	0.54	0.59	0.91	0.27	0.37	0.90
P retained, g/d	0.36	0.34	0.71	0.30	0.36	0.98

[1]Diets: Low (9.9% CP); Medium (12.5% CP); Osc: Low and High (14.2% CP) diets oscillated on a 48-h interval.
[2]SEM for n = 5.

tion as desired, there was greater variation in the P concentration among the various diets than expected, with the Medium diet having the greatest concentration (2.95 g/kg), followed by the High diet (2.80 g/kg) and the Low diet (2.60 g/kg).

The DMI of wethers fed the Medium diet did not differ from those fed either the Low ($P = 0.42$) or Osc ($P = 0.17$) diet. Apparent DM digestibility did not differ ($P \geq 0.57$) among the various treatment groups. As designed, N intake did not differ (Table 2; $P = 0.22$) between wethers fed the Medium and Osc diets, and wethers fed the Low diet consumed less N than did wethers fed the Medium diet ($P = 0.04$). All N intakes were less than the recommendations of the NRC (1985). Similarly, the amount of apparently digested N was not different ($P = 0.26$) between wethers fed the Medium or Osc diet, but wethers fed the Medium diet ($P = 0.01$) digested more N than did those fed the Low diet. Urinary N (total and as urea N) was less ($P < 0.001$) in wethers fed the Low diet than those in the other treatments. Urinary urea N was 46 to 61% of total urinary N. Although urinary N and fecal N were not statistically different ($P \geq 0.24$) between wethers fed the Medium and Osc diets, wethers fed the Osc diet retained more ($P = 0.04$) N than did those fed the Medium diet. There was no difference ($P = 0.56$) in N retention between wethers fed the Low or Medium diet. Nitrogen retention as a percentage of N digested ranged from 30 to 46%, which was somewhat greater than is typical. These large values may be due to consumption of N being below that required to support maximum growth of the wethers.

Because of the variation in dietary P concentration and DMI described above, wethers fed the Low diet tended ($P = 0.09$) to have a lower P intake than those fed the Medium diet. However, there was no difference ($P = 0.52$) in P intake between wethers fed the Osc or Medium diet, nor was apparently digestible P different ($P \geq 0.31$) among the dietary treatment groups. Additionally, there was a tendency for the wethers fed the Low diet to have less fecal P excretion than wethers fed the Medium diet ($P = 0.10$), and there were no differences ($P \geq 0.36$) in P retention among the dietary treatments.

There were limited differences among treatments in blood metabolite concentrations (Table 3). There were no differences ($P \geq 0.17$) in the blood concentrations of nitrogenous compounds (AAN, ammonia N, urea N, glutamine, and glutamate) between the wethers fed the Medium or Osc diet. Arterial ($P = 0.01$) and portal venous ($P = 0.02$) oxygen concentrations were lower in wethers fed the Medium diet than in those fed the Osc diet, but hepatic venous oxygen concentrations were not different ($P = 0.51$). There was a tendency for a lower hepatic venous ($P = 0.06$) glucose concentration when wethers in the Osc treatment group were fed the High diet than when they were fed the Low diet. There was a tendency ($P = 0.06$) for wethers fed the Low diet to have lower portal venous ammonia concentrations than those fed the Medium diet, and they tended ($P = 0.06$) to have lower urea N concentrations in all 3 blood vessels than did wethers fed the Medium diet.

There was a greater influence of dietary treatments on metabolite flux (Table 4) than on blood concentrations. Neither hepatic venous nor portal venous blood flow differed ($P \geq 0.25$) among dietary treatments, although hepatic arterial blood flow was greater ($P = 0.05$) in lambs fed the Low diet than those fed the Medium

Table 3. Metabolite concentrations of the arterial, portal venous, and hepatic venous blood of wethers fed a high-concentrate diet with low, medium, or oscillating (Osc) dietary protein[1]

Item	Treatment				SEM[2]	Contrast, *P*-value		
	Low	Medium	OsH	OsL		OsH vs. OsL	Medium vs. Osc	Low vs. medium
No. of wethers	3	5	4	4				
α-Amino N concentration, m*M*								
Artery	5.07	4.22	4.21	4.98	0.59	0.14	0.47	0.29
Portal vein	5.30	4.50	4.50	5.37	0.66	0.13	0.41	0.36
Hepatic vein	5.25	4.34	4.28	5.05	0.57	0.16	0.60	0.23
Ammonia N concentration, m*M*								
Artery	0.197	0.199	0.203	0.235	0.028	0.37	0.48	0.51
Portal vein	0.291	0.374	0.341	0.386	0.039	0.33	0.67	0.06
Hepatic vein	0.192	0.194	0.193	0.222	0.025	0.37	0.60	0.51
Urea N concentration, m*M*								
Artery	3.5	7.3	6.2	7.0	1.6	0.57	0.54	0.06
Portal vein	3.3	7.2	6.0	7.0	1.6	0.50	0.56	0.06
Hepatic vein	3.5	7.5	6.4	7.5	1.7	0.47	0.62	0.06
Glutamine concentration, m*M*								
Artery	0.247	0.253	0.213	0.249	0.023	0.23	0.27	0.84
Portal vein	0.210	0.236	0.195	0.228	0.020	0.22	0.17	0.28
Hepatic vein	0.222	0.232	0.177	0.232	0.025	0.09	0.20	0.72
Glutamate concentration, m*M*								
Artery	0.048	0.045	0.043	0.046	0.005	0.35	0.92	0.85
Portal vein	0.052	0.047	0.043	0.047	0.005	0.27	0.75	0.67
Hepatic vein	0.072	0.061	0.058	0.069	0.007	0.11	0.50	0.38
Glucose concentration, m*M*								
Artery	3.76	3.73	3.65	3.95	0.14	0.17	0.98	0.85
Portal vein	3.74	3.75	3.66	3.98	0.14	0.12	0.89	0.99
Hepatic vein	3.97	3.96	3.88	4.25	0.14	0.06	0.65	0.88
L-Lactate concentration, m*M*								
Artery	0.41	0.49	0.43	0.65	0.087	0.22	0.91	0.55
Portal vein	0.49	0.55	0.49	0.73	0.099	0.20	0.98	0.72
Hepatic vein	0.45	0.52	0.43	0.68	0.098	0.14	0.80	0.69
Oxygen concentration, m*M*								
Artery	5.46	5.25	6.02	5.77	0.16	0.15	0.01	0.29
Portal vein	4.19	4.02	4.65	4.35	0.20	0.13	0.02	0.49
Hepatic vein	3.35	3.11	3.56	3.22	0.30	0.25	0.51	0.50

[1]Diets: Low (9.9% CP); Medium (12.5% CP); Osc: Low and High (14.2% CP) diets oscillated on a 48-h interval. The Osc treatment was separated into those measurements made when the wethers were consuming the High diet (OsH) or the Low diet (OsL).
[2]SEM for the Low diet.

diet. Although not statistically significant ($P = 0.29$), AAN release from the PDV of wethers fed the Osc diet was 28% greater compared with those fed the Medium diet, and was 47% greater compared with those fed the Low diet. Consistent with the numerical changes in AAN uptake from the PDV and improved N retention, PDV uptake of urea N tended to be greater ($P = 0.06$) for the wethers fed the Osc diet than for those fed the Medium diet, although hepatic ($P = 0.72$) and total splanchnic ($P = 0.46$) urea N fluxes were not different between the 2 groups. Although there were limited differences in the net flux of nutrients through the total splanchnic tissues of wethers when they were fed the Low or High diet, there was a tendency for greater splanchnic release of glucose when wethers were fed the Low diet than when they were fed the High diet.

Similar to the effects of the Osc treatment, urea N uptake by the PDV was numerically ($P = 0.26$) greater for wethers fed the Low diet than for those fed the Medium diet (Table 4). Additionally, wethers fed the Low diet had less ammonia released from the PDV ($P = 0.05$) and taken up by the hepatic tissues ($P = 0.04$) than those fed the Medium diet. There tended to be a greater PDV uptake of glutamine ($P = 0.07$) in wethers fed the Low diet than in those fed the Medium diet, but there were no differences between the Low and Medium diets in the hepatic ($P = 0.74$) or total splanchnic uptake ($P = 0.36$) of glutamine.

DISCUSSION

Previously (Cole, 1999) and in the current study, ruminants fed high-concentrate diets demonstrated improved N retention when dietary protein was oscillated, as compared with those consuming comparable quantities of protein at a static concentration. Other studies

Table 4. Blood flow and net nutrient flux across portal-drained viscera (PDV) and hepatic tissues of wethers fed a high-concentrate diet with low, medium, or oscillating (Osc) dietary protein[1]

Item	Treatment				SEM[2]	Contrast, *P*-value		
	Low	Medium	OsH	OsL		OsH vs. OsL	Medium vs. Osc	Low vs. medium
No. of wethers	3	5	4	4				
Blood flow, L/h								
Portal venous	169	153	159	160	27	0.68	0.55	0.71
Hepatic venous	234	193	184	194	31	0.63	0.70	0.25
Hepatic arterial	66	39	26	35	13	0.67	0.81	0.05
α-Amino N net flux,[3] mmol/h								
PDV	38	41	50	55	16	0.35	0.29	0.90
Hepatic	–12	–24	–39	–46	16	0.28	0.05	0.72
Splanchnic	26	16	13	11	15	0.74	0.20	0.86
Ammonia N net flux, mmol/h								
PDV	13.9	25.3	21.3	23.6	3.9	0.41	0.51	0.05
Hepatic	–15.1	–26.4	–23.0	–25.8	3.8	0.31	0.69	0.04
Splanchnic	–1.3	–1.1	–1.7	–2.0	0.7	0.40	0.14	0.85
Urea N net flux, mmol/h								
PDV	–25	–16	–29	–24	7	0.53	0.06	0.26
Hepatic	25	51	61	48	11	0.31	0.72	0.07
Splanchnic	0	35	33	25	11	0.65	0.46	0.01
Glutamine net flux, mmol/h								
PDV	–6.7	–2.7	–2.3	–3.1	1.7	0.55	0.99	0.07
Hepatic	0.2	–1.3	–5.3	–0.7	3.9	0.16	0.56	0.74
Splanchnic	–6.5	–4.0	–7.5	–3.8	2.3	0.19	0.36	0.36
Glutamate net flux, mmol/h								
PDV	0.80	0.32	0.12	0.04	0.68	0.97	0.54	0.61
Hepatic	5.02	2.72	2.99	4.26	0.79	0.11	0.08	0.03
Splanchnic	5.83	3.02	3.10	4.29	0.83	0.11	0.21	0.01
Glucose net flux, mmol/h								
PDV	–2.5	3.7	2.5	6.5	4.7	0.24	0.50	0.28
Hepatic	54.7	40.7	37.8	51.3	9.9	0.16	0.51	0.22
Splanchnic	52.2	44.3	40.2	58.2	13.0	0.08	0.42	0.58
L-Lactate net flux, mmol/h								
PDV	14.4	8.9	10.1	12.8	3.2	0.25	0.42	0.51
Hepatic	–7.0	–3.9	–9.4	–6.3	2.6	0.17	0.04	0.77
Splanchnic	7.5	5.0	0.6	6.3	4.2	0.10	0.37	0.79
Oxygen net flux, mmol/h								
PDV	–217	–199	–206	–211	26	0.51	0.60	0.75
Hepatic	–287	–237	–221	–252	49	0.38	0.67	0.35
Splanchnic	–504	–436	–427	–464	69	0.37	0.61	0.43

[1]Diets: Low (9.9% CP); Medium (12.5% CP); Osc: Low and High (14.2% CP) diets oscillated on a 48-h interval. The Osc treatment was separated into those measurements made when the wethers were consuming the high-protein diet (OsH) or the low-protein diet (OsL).
[2]SEM for the Low diet.
[3]A positive number indicates net release, whereas a negative number indicates a net uptake.

failed to demonstrate positive benefits of oscillating dietary protein concentrations (Simpson et al., 2001; Ludden et al., 2002). A multitude of factors may explain this disparity in results, in particular, factors affecting endogenous urea N entering the gut, such as excesses or deficiencies in CP relative to the requirements of the animal. Cole (1999) hypothesized that the improvement in N retention by oscillating dietary CP could be due to improved recycling of N. That hypothesis is supported by the findings of our study, which show a tendency for greater net flux of urea N to the PDV for the Osc relative to the Medium diet, but not relative to the Low diet. Ruminants fed high-roughage diets have less carbohydrate fermentation in the rumen than those fed high-concentrate diets. The localization of N entering the forestomach, as opposed to the poststomach, is affected by carbohydrate fermentation in the gastrointestinal tract (Huntington, 1989). The increased transfer of endogenous urea to the rumen, as opposed to the hindgut, when ruminants are fed a high-concentrate diet may be due to increased numbers and activity of ureolytic bacteria adhering to the ruminal epithelium and to lesser ammonia concentrations in the rumen under these conditions (Cheng and Wallace, 1979; Kennedy et al., 1981; Javorsky et al., 1987). When ruminants are fed a high-forage diet, a greater proportion of recycled urea is taken up by poststomach tissues, but because the animal would be unable to reuse this N, there would be little improvement in N retention. Ruminants fed higher concentrate diets, which would lead to more N recycled to the rumen, would have greater potential for production of microbial protein

from recycled urea N, and the animal potentially would use this N for productive purposes. Additionally, if oscillating dietary protein improves N retention by the mechanism of altering the amount of N recycled to the gut, then an improvement in N retention it is unlikely to be apparent if the dietary protein concentrations are at or above the levels required by both the animal and the ruminal microbes. If N in the rumen exceeds microbial needs, additional N would likely have little benefit (Satter and Slyter, 1974). Additionally, it should be noted that although there was essentially no splanchnic release of urea from wethers fed the Low diet, they still excreted 2.10 g of urea N/d. A word of caution should be extended toward direct comparison of the balance data and the nutrient flux data because they were collected at different times in the animal's growth cycle and encompassed different ranges of times (4 d vs. 6 h).

Although oscillating dietary protein will likely be effective in improving N retention only at dietary protein concentrations near or below the needs of the animal, it may be an effective tool for producers to improve nutrient management. Although a disparity does exist in the literature on the effects of oscillating protein levels on N retention, there has been little depression in performance [Cole et al., 2003; Ludden et al., 2003; S. L. Archibeque, D. N. Miller (USDA-ARS, Soil and Water Conservation Research Unit, Lincoln, NE), H. C. Freetly, E. D. Berry (USDA-ARS, US Meat Animal Research Center, Clay Center, NE), and C. L. Ferrell; unpublished data] when dietary protein has been oscillated. In fact, as previously discussed, the efficiency of N utilization when ruminants have been fed oscillating dietary protein concentrations has been at least equal to, if not better than, when ruminants have been fed a static concentration of protein. Therefore, it is possible that oscillating dietary protein at a dietary N intake less than that provided by a static dietary protein concentration could yield similar performance.

Previous work in cattle has demonstrated that increasing AA flow to the small intestine—either by infusing casein into the abomasum (Guerino et al., 1991; Taniguchi et al., 1995; Krehbiel and Ferrell, 1999), by increasing DMI (Huntington et al., 1988; Glenn et al., 1989), or by supplementing the diet with protein (Krehbiel et al., 1998)—increases the net portal release of AAN. Although a statistical difference was not found in our study, wethers fed the Osc diet had a numerically greater PDV release of AAN than did wethers fed the Medium diet, and this difference in PDV AAN release mirrored treatment effects on N balance, with those fed the Medium diet having only a negligible improvement in N retention compared with those fed the Low diet, and those fed the Osc diet having a significant improvement in N retention compared with those fed the other diets. It should also be noted that wethers fed the Osc diet had a numerically greater N intake than those fed the Medium diet, which may have contributed to the increase in AAN release from the PDV. The difference in N retention between the Osc and Medium diet-fed wethers (2.7 g/d) equates to 8.0 mmol of N retained/h, and the numerical difference in AAN PDV release was 11.5 mmol/h, indicating again that, although not statistically different, the increase in PDV release of AAN was sufficient to support the observed increase in N retention. Although the PDV AAN release followed the same numerical trend as N retention, hepatic AAN uptake also was increased, such that the net splanchnic AAN release was reduced. This may be partially explained by noting the relationship between the total amount of AAN and ammonia taken up by the hepatic tissues and the subsequent release of urea by the liver. The sum of AAN and ammonia taken up by the liver ranged from 27.5 mmol/h in wethers fed the Low diet to 67.3 mmol/h in wethers fed the Osc diet. Except for the Medium group, the quantity of N taken up by the liver, as AAN and ammonia, was greater than the N released as urea N by the liver. This is similar to previous studies in sheep (Krehbiel et al., 1998; Ferrell et al., 1999) and cattle (Eisemann et al., 1996) in which urea N release from the liver accounted for 42 to 100% of the sum of AAN and ammonia N taken up by the liver. This result is in contrast to several studies with beef cattle (Reynolds and Tyrrell, 1991; Reynolds et al., 1991, 1992; Taniguchi et al., 1995), which reported that urea N release from the liver accounted for 98 to 130% of hepatic removal of AAN and ammonia N. Additionally, when deproteinized plasma samples were used to determine nutrient flux, urea N release from the liver accounted for 130 to 150% of the sum of AAN and ammonia N taken up by the liver (Savary-Auzeloux et al., 2003). The small amounts of AAN released by total splanchnic tissues suggests that ruminants deliver AAN to extrahepatic tissues in a form other than free AAN. Connell et al. (1997) demonstrated that nutritional alterations (fasting vs. supra-maintenance intake) can alter the amount of hepatic venous free AA and total plasma albumin content. Additionally, Connell et al. (1997) found a greater amount of apolipoprotein B100-bound AA than was present in the liver homogenate of fed wethers. We speculate that AA may be transported as constituents of blood proteins (i.e., albumin, fibrinogen, lipoproteins) or possibly bound to the surface of large proteins, effectively "masking" the α-amino group. This binding of numerous metabolites and drugs has been well documented in the literature, as have the various rates and mechanisms of transport to and from such molecules (Tanford, 1981; Weisiger et al., 1981; Weisiger, 1985). Limitations involved with the measurement of AAN as opposed to individual AA may contribute to the low splanchnic release of AA to support growth of the wethers. Additionally, it should be noted that AAN does not reflect the profile of AA being released or absorbed and could be influenced, in particular, by alterations in the flux of nonessential AA, such as glutamine.

There has been a great deal of interest in the "first pass" metabolism of AA by the small intestine. Both glucose and glutamine are important metabolic fuels

for gut tissue (Windmueller and Spaeth, 1980; Okine et al., 1995), and recent studies have focused on the importance and substitutive properties of these fuels in nonruminants (Wu et al., 1995; Stoll et al., 1998; Wu, 1998) and ruminants (Okine et al., 1995; Oba et al., 2004). Oba et al. (2004) distinguished a "different extent of reliance on glutamine as an energy source between ruminant and nonruminant enterocytes." (p. 485). This was largely based on the view that glutamine oxidation is spared in the presence of other metabolic substrates and that the extent of sparing may be concentration dependent for some substrates, such as propionate. Additionally, El-Kadi et al. (2006) demonstrated that the extent of metabolism of AA, especially that of branched-chain AA and certain nonessential AA, by gastrointestinal tissues will vary with alterations in the level of intestinal supply of protein. In our study, when wethers were fed the Low diet, they tended to have a greater PDV uptake of glutamine than when they were fed the Medium diet. This was likely due to an insufficient amount of glutamine being absorbed from the lumen of the small intestine. However, Doepel et al. (2005) noted no improvement in either energy or protein metabolism across the gut of lactating dairy cows that were supplemented with 300 g of glutamine/d. This may indicate a specific need by the intestinal mucosa for a basal amount of glutamine that was already supplied by the diets of these cows fed a standard dairy total mixed ration. However, this disparity in the literature warrants more research to truly elucidate the role of glutamine in the "first-pass" metabolism of ruminant enterocytes, which may comprise a substantial portion of AA catabolism within the animal.

Our experiment offers evidence that at least a portion of the mechanism for improved retention of dietary N by ruminants fed oscillating protein diets may be due to an increased uptake of urea N to the PDV. Unfortunately, because of the lack of sensitivity (n = 5 for the Medium diet and n = 8 for the Osc diet) with the measurement of the net flux of nutrients across splanchnic tissues, it is impossible to state this definitively. This is particularly true because the changes observed in N retention were less than the error associated with our measurement of PDV AAN release. Moreover, our study did not investigate the potential for coordinated alterations in rumen microbial populations or functionality. For the feeding of oscillating concentrations of dietary protein to become truly viable for a finishing animal system, overall dietary protein intake likely will have to be at or slightly less than the requirements of the animal. Although this is an unlikely scenario in many current feedlot systems, which typically feed nutrients at a concentration greater than required for adequate growth of the animals, it may provide a potential tool for producers to more closely manage nutrient loss into the manure.

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